VIII PLAN PROJECT
VERONICA RODRIGUES

Genetic analysis of sensory coding in the chemosensory pathways of *Drosophila melanogaster*.

(1990-1995)

Staff:

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Introduction

Our broad objective is to understand how information about chemical signals is encoded and processed in the central nervous system of *Drosophila*. What are the molecules that specify the development of the underlying circuits and how is the function of these circuits specified? These question can be posed at two levels: 1. What makes a cell differentiate into a receptor neuron selectively tuned to a small subset of chemical stimuli. 2. What are the factors that decide the meaning the afferent signals convey to the brain. Both these questions are necessarily developmental and require a knowledge of how peripheral neurons develop and connect to the appropriate targets in the central nervous system. Genetic and molecular studies in a number of laboratories have elucidated the role of the neurogenic loci in the early developmental decisions that lead to differentiation of epidermal cells into either peripheral neurons or cells of the central nervous system (Ghysen and Dambly-Chaudiere, 1989). The processes that control the specialization of sub-populations of neurons to perform specific processing tasks are far less well understood. Studies on the nervous system of the fly centre around the visual system. Of note here, are the genes *sevenless*, *bride of sevenless* and *rough* which provide a handle for the study of interactive influences in the development of the photoreceptor cells (Ready ,1989).

The chemosensory system of *Drosophila* is amenable to a study of how genes interact with the environment to result in specific kinds of cells. The majority of taste sensillae, located on the proboscis are innervated by four different neurons-one responsive to sugars, two to salts and one to water. A smaller subset of taste hairs are innervated by either two or three neurons. The pattern of innervation and the sensitivities of the neurons are invariant from individual to individual and are is therefore likely to be under strict genetic control. As yet little is known about the circuits to which these

receptors wire, but the behavioral responses that can be measured suggest several levels of integration in the brain. A number of mutations have been isolated that lead to strong behavioral changes suggestive of possible central defects.

In the olfactory system, functional mapping experiments have shown that each odorant excites a specific subset of neurons in the antennal lobe; the subsets are overlapping, but distinct for each odor class (Rodrigues, 1988). This means that there exists in the brain of the fly a spatial map for each odor quality. This proposal raises several interesting questions about information processing. For example, one can ask, if there are pre-determined sensory maps in the brain, to which incoming neuronal activity must be matched to give rise to perception. Are these maps genetically hard-wired or does plasticity during development play a significant role? These are some of the issues that we wish to address using genetic and molecular methods.

The ability to subject *Drosophila* to a genetic analysis -viz isolation of behavioral mutants, their characterization and subsequent molecular analysis of the identified gene is well known. We aim to continue this approach in the chemosensory system. The drawback of this approach, however, is that it often requires the assumption of a specific phenotype. Genes that have roles in several tissues or at several stages of development can be missed in screens that select for phenotypes of a very specific nature. This is of importance in Drosophila where a large number of genes have functions early in development that mutate to lethality.

A recently described approach, the enhancer-trap scheme, overcomes some of these drawbacks (O'Kane and Gehring, 1987). Briefly, this strategy involves the mobilization of a P-element tagged to a reporter gene,beta-galactosidase ,to different positions on the fly's genome. The weak but constitutive promoter of the transposon comes under the influence of enhancers in the vicinity of its insertion. The spatial and temporal distribution of beta-galactosidase activity reflects the expression of the 'trapped' gene. The availability of probes for the inserted sequence makes mapping as well as cloning of the genes relatively simple and much more rapid compared to conventional methods.

Review of seventh plan period (1985-1990)

In the last plan period we had two broad objectives which will be discussed below.

1. To identify genes that could be candidates for a role in the function/development of the circuits processing chemosensory information.

We followed three main approaches for the identification of so-called "central" genes.

1.1 Classical approach.

This involved isolation and characterization of mutations whose phenotypes fitted with 'pre-conceived' ideas about what kind of behavioral changes a defect in circuitry would have. Our working definition of a central mutant, for both olfaction and taste ,was one in which the resulting lesion had a effect on the perception of several different chemical stimuli i.e. were pleiotropic. In the olfactory screens we used different types of enrichment and testing protocols to isolate five P-induced and four EMS -induced mutants which are being analyzed in detail.

We have also analyzed a set of gustatory mutations - *gustB, gustC, gustG* and *gustD*. *gustB* and *gustD* show specific alterations in behavior to sodium chloride and quinine respectively, while the other mutations cause pleiotropic defects affecting sensing of sugars, salts and quinine.The behavioral aberration in *gustB* has been explained by a mis-expression of receptors on the peripheral neurons (Arora et al, 1987). *gustG* and *gustD* do not show any detectable peripheral lesions. The receptor neurons in *gustC* are hyperexcitable to sodium chloride, but do not show alterations in response to sugars. We speculate that this gene acts at peripheral and central levels in the taste pathway. The loci map to a four band region (10E1-4) and show complex interactions in trans. The possibility that these loci describe a complex locus is being investigated.

1.2: Effects of other neurological mutants on chemosensory behavior

Studies on genes like *daughterless* have established the point that the same molecule can serve in very different developmental and functional roles (Caudy et al, 1988). We looked among mutations which are known to affect formation of circuits or synapses for alterations in chemosensory behavior. Mutations in the gene *shakingB* which was known to affect synapses in the pathway mediating the light-evoked jump response also affect taste behavior. This suggests a more general role for *shaking B* in development of taste circuits in addition to the jump pathway. Our detailed genetic analysis

of this locus leads us to suggest that the *shaking B* gene interacts *in trans* with the neighboring complementation group R-9-28, to encode taste function.

The *Shaker* locus in *Drosophila* has been shown to encode a family of A-type potassium channels. As many as 20 different transcripts are generated from this locus by alternative splicing of mRNA from this locus (Timpe et al.,1988). Cellular heterogeneities in localization of these transcripts can, in principle, generate neurons with vast diversities in signalling and modulatory properties. We have examined the gustatory responses of a set of *Shaker* alleles with a view to dissecting the taste pathway of *Drosophila* In our view of the coding of taste information, attraction input is weighed against repulsion to generate behavior. One possibility is that all repellents share a common labelled line. In this case mutations increasing tolerance to KCl would simultaneously result in an enhanced tolerance to high concentrations of NaCl. Our results with the *Shaker* alleles suggest that the responses to NaCl and KCl are, in fact, uncoupled. The fly must therefore posses an information channel that allows it to discriminate between NaCl and KCl.

1.3: Trapping of enhancers of chemosensory genes.

We have recently initiated a project in collaboration with Drs. K. **VijayRaghavan** and **Gaiti Hasan** to use the newly described enhancer trap scheme to identify genes which express selectively in the chemosensory pathways. From several hundred lines each containing such a mobilized element, we identified four autosomal and one X- linked lines whose pattern of staining suggests the identification of genes which play a role in the chemosensory system. These lines are being analyzed using genetic and molecular techniques.

2. Analysis of normal olfactory function and development.

We used immunocytochemical, histological and mutational approaches to follow the development of the adult antennal nerves from their origin in the antennal disc to the brain. The adult antennal nerve arises from the antennal part of the cephalic disc and enters the brain in close proximity with the existing larval olfactory nerve. During pupation, the antennal lobe increases in size and assumes a morphological division into 22 synaptic clusters or glomeruli. The development of the glomeruli is dependent on the arrival of afferent fibres from the antennae.

The organization of the antennal lobe into distinct anatomical areas has profound implications in odor coding. We had previously shown that odor quality is encoded by a spatial representation of neuronal activities among the different glomeruli. How is the spatial map for each odorant generated? One possibility is that the sensory map reflects a precise connectivity of receptor neurons of determined specificity to distinct subsets of glomeruli. On the other hand, it is possible that the spatial activity domains arise, not only from the input information, but by complex excitatory and inhibitory influences of the interneurons in the antennal lobe. We examined this question by visualizing the affect on the sensory map of stimulation by mixtures of odors, rather than by pure chemicals. Our preliminary results lead us to postulate that while the receptor neurons dictate the bulk of the sensory map, this is tuned to a significant extent by excitatory and inhibitory interactions between the antennal lobe interneurons. The interpretation of these findings will remain speculative until these results can be analyzed in light of a detailed synaptology of the antennal lobe.

Projects for the eight plan.

1. Genetic analysis of gustatory mutants which may affect central pathways.

Several autosomal mutations have been isolated by Swati Sathe, Kavita Arora and O. **Siddiqi** which lead to strong pleiotropic defects in taste behavior. One of these, *GustR* has been studied in some detail. The peripheral receptor firing in these flies is defective in response to salts, but normal to sucrose. This lesion is insufficient to explain the behavioral deficit. One possibility is that this gene is involved at both central and peripheral levels.

Rohini Balakrishnan has used overlapping deficiencies as well as ry+ insertions in the vicinity to map this locus to 64B-C on the third chromosome. A strain bearing a ry+ insertion in this region has a phenotype comparable to *GustR*. The dominant nature of this mutation, however makes it difficult to ascertain whether the insertion is in fact allelic to *Gust R*. The availability of insertions flanking *GustR* makes it possible for us to search for deficiency alleles of this locus. Studies on the null phenotype will enable us to infer the normal role of this gene.

We will begin a large scale mutagenesis to look for *GustR* alleles and revertants using P-inserts . In these experiments, a strain containing a modified vector will be used for mutagenesis (Cahir O'Kane, Norwich, U.K.) . The vector contains, in addition to the P-lacZ construct, a bacterial origin of replication and an ampicillin resistance marker, both of which facilitate the

rapid cloning of DNA flanking the insert. These screens will be designed so that mutants in other, previously unidentified genes will also be selected for. The advantage of obtaining mutants induced by P(lacZ,ry+) is that the position of the mutated gene on the chromosome as well the pattern of its expression can be readily studied.

Other strategies for cloning *Gust R* will be walking to the gene using nearby clones as entry points or use of P(ry+) inserts to specifically amplify flanking DNA using the polymerase chain reaction. Availability of the cloned gene will allow us to study its spatial and temporal expression and its possible role in development of taste circuits.

2. Identification and characterization of genes expressing in the chemosensory pathways enhancer trap schemes.

2.1 Autosomal genes.

Of several hundred lines screened, we have selected four lines in which the pattern of beta -galactosidase staining suggests that the native gene plays an important role in the development of the chemosensory pathway. We plan to carry out a detailed developmental, genetic and molecular analysis of these genes. The cloning of these genes will be carried out using the polymerase chain reaction to amplify regions of DNA flanking the insert. This DNA will be used as probes to screen a genomic library from wildtype flies.
The genes can be readily mapped by in-situ hybridization using cloned probes of the mobilized elements. Excision of the elements using 'jumpstart' strains and deletion of the flanking DNA by X-ray irradiation will be used to generate mutations in the region and their effects on behavior studied.

2.2 X-Linked genes.

One hundred lines with P(lacZ,ry+) inserts on the X chromosome were set up by Joyce Fernandes (K. VijayRaghavan's group) and A. Anand (IISc. Bangalore). These lines have an advantage that the insert contains a bacterial origin of replication and an ampicillin resistance gene, both of which make cloning of the flanking DNA relatively easy. Three lines have so far been identified which show beta-galactosidase staining in the taste and olfactory sensilla and in very specific regions of the central nervous system. In one of these lines, the insertion has resulted in the mutation of a previously identified gene *scalloped* which results in a defective wing phenotype. This insertion simultaneously leads to aberrations in taste and olfactory behavior. This locus will be characterized in detail.

The autosomal and the X chromosome lines will all be tested in behavioral assays to screen for mutants showing pleiotropic defects in olfaction and/or taste.

3. Development of the chemosensory pathways.

We plan to use immunocytochemical and genetic methods to trace the development of the sensory neurons in the adult from the imaginal discs to the brain of *Drosophila*. These studies will be aided by an analysis of P(lacZ) insert lines that stain all sensory neurons. The influence on the pattern of expression of mutants in genes known to be involved in peripheral neurogenesis and/or result in behavioral deficits will be examined. We plan to use these lines as well as other markers to carry out a clonal analysis of the sensory neurons in the taste sensillum. Special attention will be given to the identification of factors which decide what makes a specific kind of neuronal cell.

We plan to continue functional mapping using 2-deoxyglucose autoradiography in the olfactory pathway of *Drosophila*. The information coding system for acetate and aldehyde odors at the level of the first synaptic relay station is now beginning to be understood. How is this information dealt with at centers post-synaptic to the antennal lobe? We plan to adapt the 2-deoxyglucose method to examining stimulus-induced activity at the higher centers in the mushroom bodies and the protocerebrum. These high resolution autoradiographic methods will be coupled with transmitter localization studies to determine the functional nature of the synapses identified. Immunocytochemical experiments in Erich Buchner's laboratory have identified a small number of serotoninergic neurons among the antennal lobe cells. We plan to follow the stimulus specificities of these neurons using 2-DG autoradiography.

Summary

In the eight plan period we forsee an increasing amount of collaboration with Dr. K. VijayRaghavan's group on studying the molecules involved in the development of the nervous system. We plan to continue, in addition, a neurogenetic approach to identifying the genes that specify the neural basis of chemosensory behavior.

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Publications

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Rodrigues, V. and Pinto, L., (1989). The antennal glomerulus as a functional unit of odor coding in *Drosophila melanogaster*. Neurobiology of sensory systems. R.N.Singh and N.J. Strausfeld(Eds.). pp 387-396. Plenum Press, new York

Budget

Capital

1. Accessories for Carl Zeiss microscope camera, DIC filters, florescence attachment	1.0 lacs
2. Dissection microscopes	0.7 lacs
3. Power supplies and electrophoresis setups	0.8lacs
4. Eppendorf Centrifuge	0.6lacs
5. Pipette aids and dispensers	0.6lacs
6. Water baths	0.3lacs
7. Inverted microscope with phase attachment	2.5 lacs
8. Micromanipulators	2.0 lacs
9. Small equipment	0.7 lacs
10. Oscilloscope, Amplifier	1.2 lacs
Total	**10.4 lacs**

Consumable

Enzymes	0.7lacs/yr
Radiochemicals/biochemicals	0.65lacs/yr

Films and emulsions	0.5lacs/yr
Immunochemicals	0.5lacs/yr
Media	0.3lacs/yr.
plasticware	0.25lacs/yr
Glassware (capillaries etc)	0.15lacs/yr
total	**3.05 lacs/yr**

15.25 lacs

Grand Total: Capital + Consumable 25.65 lacs

Projected staff.

Our group at present consists of two and a half doctoral students (one is shared with K. VijayRaghavan) and one scientific officer. We envisage adding at least two students (Ph.D. or M.Sc.) and two post-doctoral workers during this plan period.

List of Publications.

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